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Development of *Encarsia bimaculata* (Heraty and Polaszek) (Hymenoptera: Aphelinidae) in *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae) nymphs☆

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Abstract

Encarsia bimaculata was recently described from India as a potentially useful parasitoid of *Bemisia tabaci*. Its developmental biology was studied in the laboratory at 25–30 °C and 70–75% RH. Results showed that *E. bimaculata* is a solitary, arrhenotokous, heteronomous, autoparasitoid. Mated females laid eggs internally in *B. tabaci* nymphs that developed as primary parasitoids. Males developed as hyperparasitoids, either in females of their own species or in other primary aphelinid parasitoids. Superparasitism was common under cage conditions. Both sexes have an egg, three larval instars, prepupal, and pupal stages. Development from egg to adult took 12.70 ± 2.10 days for females and 14.48 ± 2.60 days for males. Individual *B. tabaci* nymphs were examined for *E. bimaculata* parasitization using three isozymes: esterase, malate dehydrogenase, and xanthine dehydrogenase. All three isozymes showed differential banding patterns that identified *E. bimaculata* parasitized or unparasitized *B. tabaci* nymphs.

Keywords: *Encarsia bimaculata*; *Bemisia tabaci*; Developmental biology; Isozymes

1. Introduction

Sweetpotato whitefly, *Bemisia tabaci* (Gennadius), is considered an international pest and a serious threat to the production of many agriculture and horticulture crops throughout the world (Brown, 1994; Butler and Henneberry, 1994; Byrne et al., 1992). Recent studies suggest that *B. tabaci* is either a complex of sibling species, or biotypes (Brown et al., 1995a; Perring, 2001). *B. tabaci* was first reported in 1905 as a serious pest of cotton and vegetables in India (Misra and Lambda, 1929). Lisha et al. (2003) identified two *B. tabaci* biotypes from India. Palaniswami et al. (2001) identified *Encarsia* spp. and *Eretmocerus* spp. parasitoids in India that have potential for suppression of *B. tabaci* populations. Effects of some of these parasitoids in *B. tabaci* control programs have been reviewed (Gerling et al., 2001; Kirk et al., 2000).

Encarsia bimaculata (Heraty and Polaszek) was first described from India by Heraty and Polaszek (2000) as a *B. tabaci* parasitoid in the 'strenua group' (Aphelinidae: Hymenoptera) that has 40 or more species. Detailed studies of its biology and parasite efficiency have not been reported. Efficient mass rearing and parasitoid releases depend on extensive knowledge of the biological relationships between the host and the parasitoid. Developmental traits of a parasitoid are an important part of the essential knowledge base. Assessment of *B. tabaci* biological control programs with parasitoids is laborious. Conventionally, parasitism percentages are determined by dissections of nymphs sampled from the population or visual observations for the occurrences of black parasite pupae. The methods are tedious and time consuming,

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because *B. tabaci* nymphal stages are small, measuring about 0.5–0.7 mm. Alternative methods proposed for identifying internal parasitoid occurrence are electrophoretic detection and ELISA gut content analysis.

The objectives of the current study were to determine the developmental biology of *E. bimaculata* to facilitate further progress in implementation of the parasitoid into a *B. tabaci* control program. We also evaluated isozymes by electrophoresis for identifying *E. bimaculata* parasites in *B. tabaci* nymphs.

2. Materials and methods

Encarsia bimaculata were collected at Coimbatore, South India, from *B. tabaci* pupae on cotton. The parasites thereafter were cultured at the Central Tuber Crops Research Institute (CTCRI) on *B. tabaci*. Dr. J.M. Heraty (University of California, Riverside, CA) verified species identification and voucher specimens have been deposited in the Division of Crop Protection, Central Tuber Crop Research Institute (CTCRI), India.

2.1. Host and parasitoid cultures

At CTCRI *B. tabaci* colonies were maintained in a screen house within iron frame cages of 70 cm (height) × 42 cm square angle covered with organdy cloth on three sides. Transparent plastic sheets covered the remaining side and the cage top. Temperatures in the screen house ranged from 25 to 30 °C, with 70–75% RH.

Bemisia tabaci parasitized pupae from the field were placed in petri dishes with 10% honey as adult parasitoid food following emergence. Emerged adult parasitoids were released in cages containing cassava plants infested with *B. tabaci* nymphs. *E. bimaculata* parasitized black pupae were sexed as females when meconium was located on the central periphery of *B. tabaci* puparia cases (Fig. 1S). For males, additional meconium occurred at the posterior periphery of the puparia (Fig. 1S). Virgin female parasitoids were obtained by confining female black pupae in vials. Emerged adult female parasitoids were placed on detached cassava leaves with abundant *B. tabaci* nymphs for about 6 h. Leaf petioles were wrapped in moist cotton to retain leaf turgidity. Parasitoids obtained sufficient nutrients for egg laying by feeding on honeydew and body fluid of whitefly nymphs, which oozed out due to ovipositor punctures. Mated female parasitoids were obtained by confining one newly emerged female with two males in petri dishes for about 6 h. Two-day-old parasitoid females were used in all experiments.

2.2. Life history and development of E. bimaculata

Approximately 50 adult *B. tabaci* were released into leaf-clip cages (4-cm diameter) attached to cassava leaves. After 24 h, adults were aspirated from the leaf-clip cages and the perimeters of the leaf-clip cages marked with indelible ink. The plants were placed within insect-proof cages until the *B. tabaci* nymphs developed to third and early-fourth instars. Leaf-clip cages were replaced and three *E. bimaculata* mated females were introduced into each cage with *B. tabaci* nymphs. After 48 h, leaf-clip cages with parasitoids were removed. Each day thereafter, randomly selected *B. tabaci* nymphs were dissected until egg, first, second, and third instars of *E. bimaculata* were obtained (~7–8 days), and stages of parasitoid development recorded. Developmental time was counted from the day the female parasitoid was released into the leaf-clip cages with *B. tabaci* nymphs. All stages of parasite development were photographed using a Leica M10 stereomicroscope equipped with an image analyzer. A total of 600 *B. tabaci* nymphs were exposed to *E. bimaculata* parasitoids. Twenty to 30 nymphs were observed for each parasitoid stage, developmental times were recorded, and each experiment was repeated six times.

Male developmental times were determined by releasing two unmated *E. bimaculata* females into leaf-clip cages with "dry environment" third instars, prepupae, and early black pupae of *E. bimaculata* and prepupae and early black pupae of *Encarsia transvena* (Timberlake) [= *Encarsia sophia* (Girault)]. The "dry environment" occurs after feeding and development of female third instar parasitoids depletes the contents of *B. tabaci* pupal cases (Antony et al., 2001; Hunter, 1989; Hunter and Kelly, 1998). The dry host environment occurs during the parasitoid prepupal and pupal stages. We chose these *B. tabaci* developmental stages because earlier cited work suggested that unmated female parasitoids preferred to oviposit unfertilized eggs in *B. tabaci* nymphs in "dry environments." Twenty to 30 individuals of each of the five different development stages listed were exposed to newly emerged, unfertilized female *E. bimaculata* for 48 h. Thereafter, female parasitoids were removed. After 24 h, the five different host stages were collected and kept separately on microscopic slides in petri dishes. Instar developmental times and photographs for the different male parasitoid stages were recorded as described above. Morphometrics of different stages of female and male parasitoids were taken using a Leica compound microscope and means of six replications are presented.

All experiments were repeated six times in a screen house under the described conditions. Cassava plant varieties used in the experiments were *M4* and *H226*.

2.3. Electrophoretic detection of E. bimaculata parasitized and non-parasitized B. tabaci nymphs

Parasitized *B. tabaci* nymphs were obtained using leaf-clip cage methods on cassava leaves as previously

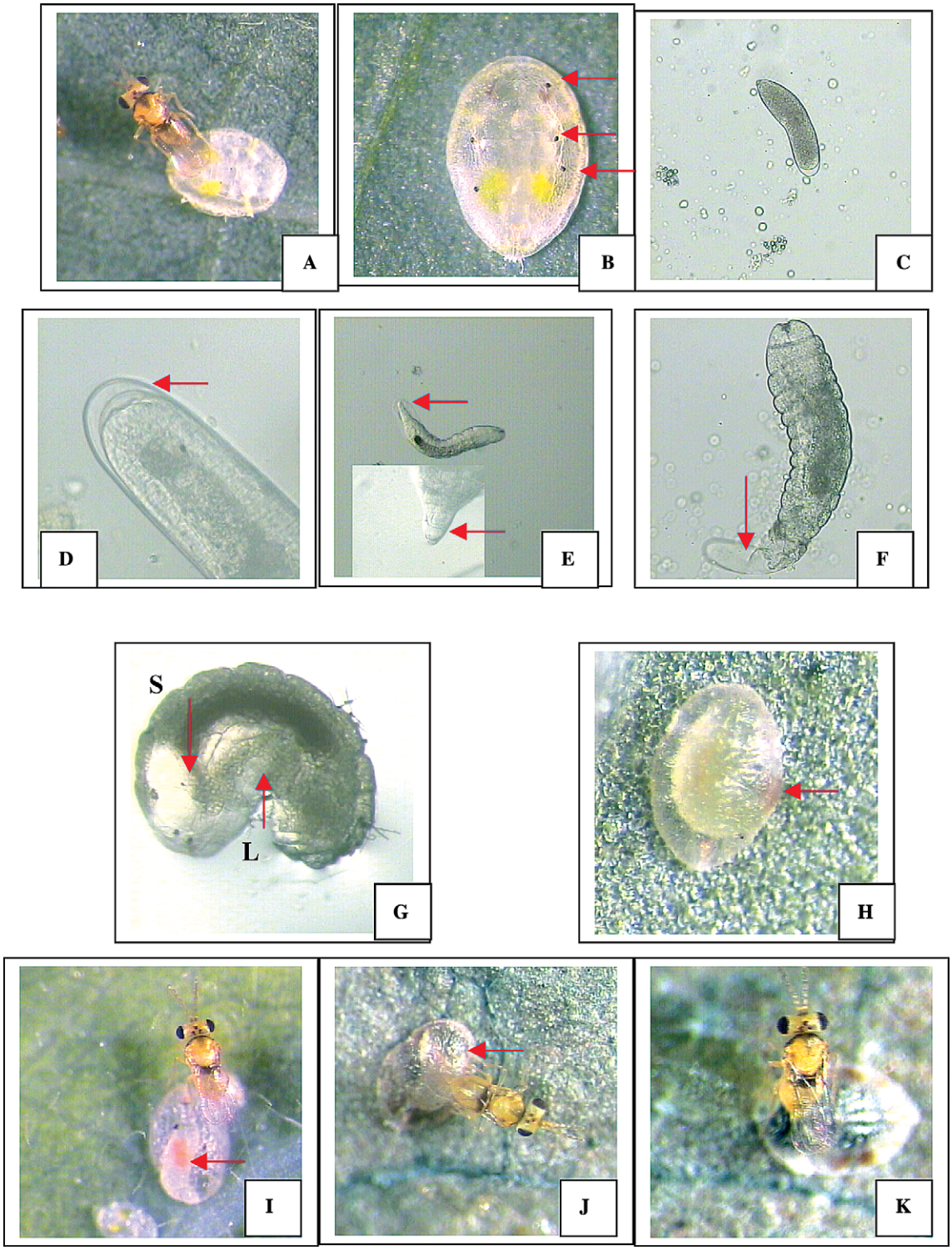

Fig. 1. (A–W) Development of female and male *Encarsia bimaculata*: (A) *E. bimaculata* laying female egg; (B) ovipositional puncturing; (C) female egg; (D) developing embryo showing cleavage nuclei and three-layered egg membrane, arrow—extraembryonic membrane; (E) first instar, arrow—tail, (inset: tail enlarged view); (F) second instar, arrow—exuviae; (G) third instar larva, S: spiracle; L, ileolabial gland; (H) prepupa laying meconium, arrow—meconium; (I) virgin female laying unfertilized egg on *E. bimaculata* female third instar inside the whitefly puparium, arrow—female third instar; (J) virgin female laying unfertilized egg on *E. transvena* female prepupa, arrow—*E. transvena* female prepupa; (K) virgin female laying unfertilized egg on *E. bimaculata* female early black pupa, arrow—female early black pupa; (L) male egg on third instar (dry environment), arrow male egg; (M) male egg on prepupa, cut open from the host puparium, ME, male egg; Me, meconium; (N) first instar (arrow); (O) second instar (arrow); (P) third instar (arrow); (Q) third instar (arrow) consumes the host; (R) prepupa, M, male; F, female; (S) black pupa, M, male; F, female, (inset: *E. transvena* black pupa); (T) adult *E. bimaculata* prior to emergence; (U) male and female *E. bimaculata*; (V) hyperparasitized male again parasitized by another *E. bimaculata*, ME, male egg; Me1, meconium female *E. bimaculata*; Me2, meconium male *E. bimaculata*; and (W) *E. bimaculata* black pupa showing three meconium, Me1, meconium of female *E. bimaculata*; Me2, meconium first male *E. bimaculata*; and Me3, meconium second male *E. bimaculata*.

described. Unparasitized nymphs were controls. Esterase, malate dehydrogenase (MDH), and xanthine dehydrogenase (XDH) enzyme systems were used in vertical slab native polyacrylamide gel electrophoresis tests. Individual *B. tabaci* nymphs were homogenized in microfuge tubes in 15 L of 0.05 M Tris–HCl (pH 6.8) containing 10% sucrose and 0.1% Triton X-100. The homogenates were transferred into wells of a stacking

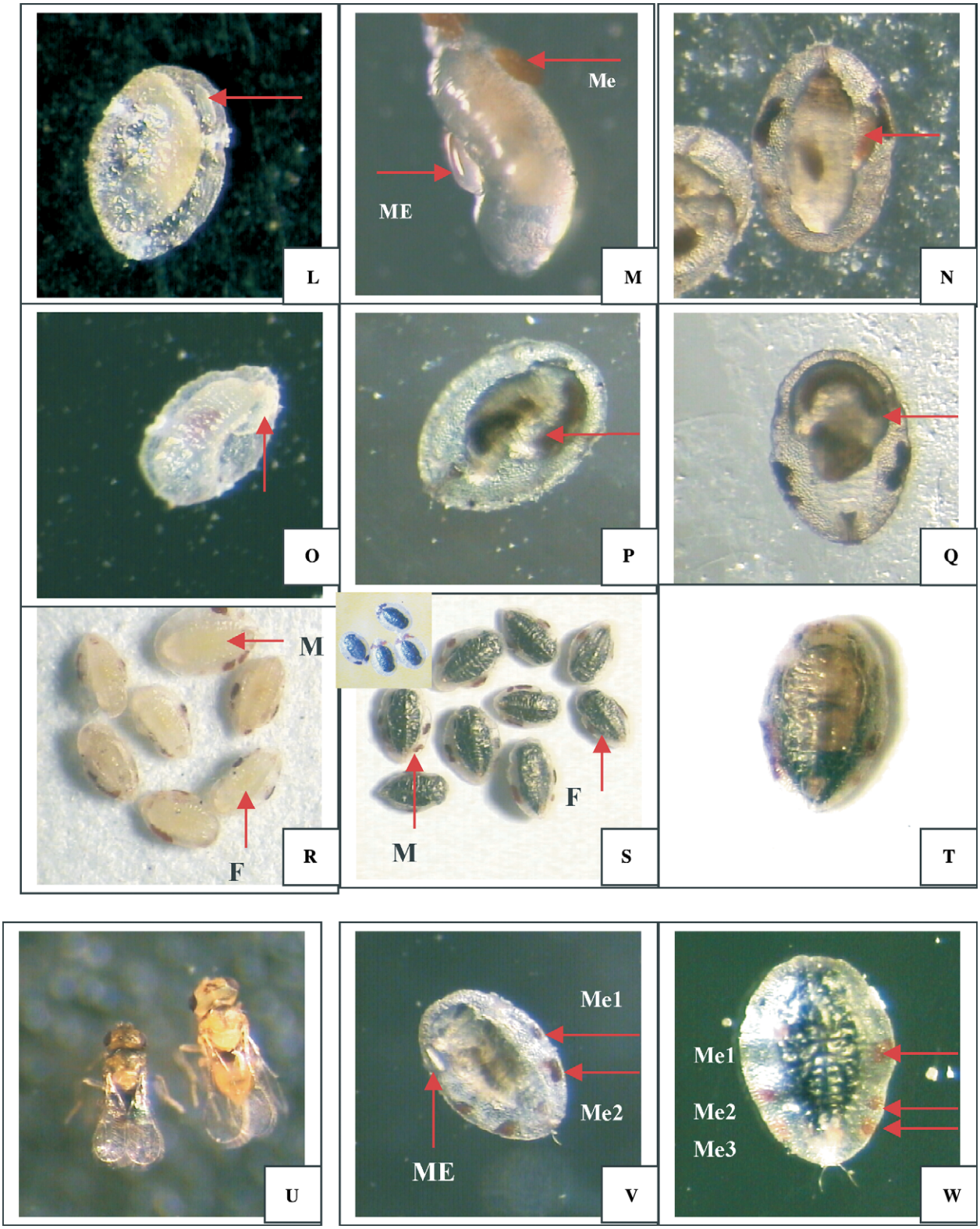

Fig. 1. (*continued*)

gel (2.5%), superposed on a 10% resolving gel. The reservoirs contained Tris 0.06 M–glycine 0.37 M buffer (pH 8.2). Gels were run at 15 mA for 15 min and then at 19 mA for 120 min. Bromophenol blue was used as the running marker. Esterases were visualized by the addition of 0.05% 1-naphthyl acetate dissolved immediately prior to use in 0.2 M phosphate buffer (pH 6.6) containing fast blue BB salt (100 mg/100 ml). MDH was visualized by immersing the gel in 1 M sodium malate solution (pH 7.5) (3 ml/100 ml) in 0.1 M Tris–HCl buffer (pH 7.5) containing NAD^+ (30 mg/100 ml), phenazine methosulfate (PMS) (4 mg/100 ml), and 4-methylthiazolyl tetrazolium bromide (MTT) (20 mg/100 ml). XDH was visualized by immersing the gel in 50 mM Tris–HCl buffer (pH 7.5) containing hypoxanthine (700 mg/100 ml), NAD^+ (30 mg/100 ml), phenazine methosulfate (PMS) (4 mg/100 ml), and 4-methylthiazolyl tetrazolium bromide (MTT) (20 mg/100 ml). Gels were photographed and analyzed using the Gel Documentation System (Bio-Rad Laboratories, California).

3. Results

3.1. Life history and developmental biology

Mean *E. bimaculata* developmental times from egg to adult at temperatures ranging from 25 to 30 °C and 70 to 75% RH were 12.7 ± 2.1 days for females and 14.5 ± 2.6 days for males. The developmental stages were egg, three larval instars, prepupa and black pupa (Fig. 1).

Female parasitoids stand over host bodies and penetrate dorsal cuticles with their ovipositors to deposit eggs (Fig. 1A). Oviposition punctures were at first light brown, but later darken (Fig. 1B). Multiple oviposition punctures occur on the same host puparium (Fig. 1B). More than one egg and emerging first instar may occur in the same host, however, only one second-instar parasitoid was found in the same host. The first emerged instar parasitoid appears to suppress the development of additional parasitoids. Eggs (Fig. 1C) averaged 0.168 ± 0.002 mm long and 0.026 ± 0.015 mm wide. Polar bodies were visible in the newly laid eggs. Egg development to the first instar occurred in 2–3 days. During this time the egg contents changed. Migrating cleavage nuclei were observed in the peripheral anterior region of the egg (Fig. 1D). Three-layered egg membranes were visible in the developing embryo (Fig. 1D). First instar parasitoids were transparent, 0.129 ± 0.097 mm long and 0.052 ± 0.015 mm wide with 3 thoracic and 10 abdominal (Fig. 1E) segments. During early first instar development the segments were not clearly visible. Larvae moved actively within the body fluid of the host. During late first instar stage, by close observation of the parasitoid under a stereomicroscope (800× magnification) and by keeping first instar larvae in insect-ringer solution, we counted the body segments. Larval head capsules were notched and contained dark-gray colored, sickle-shaped mandibles. Larvae moved actively within the body fluid of the host. The first instar has 0.041 ± 0.012 mm long tail (Fig. 1E). Developmental time from first to second instar was 2 days. The second instar measured 0.267 ± 0.910 mm long and 0.052 ± 0.019 mm wide. Segment 13 had a small button-like structure. Most of the internal organs were visible through the transparent cuticle (Fig. 1F). Development from the second to third instar occurred in 2 days. In the late second instar, remnants of the cast skin were observed loosely attached to the last abdominal segments of larvae (Fig. 1F). The third instar was 0.487 ± 0.130 mm long and 0.114 ± 0.009 mm wide (Fig. 1G). Nine pairs of spiracles, first two pairs occurred on second and third thoracic segments and remaining on first seven abdominal segments (800× magnification) (Fig. 1G). Third instar larvae completed development in 2 days. During this interval larvae depleted the body contents of the host body ("creating a dry environment"). Yellow-colored gut content changed to light brown and was visible through the *B. tabaci* nymphal cuticle. Larvae were sickle-shaped and the ileolabial gland was visible through the ventral side of the larvae (Fig. 1G). Third instar larvae moved to the anterior wall of the host puparia. Third instar larvae positioned in the central peripheral area of the host produced meconia pellets (Fig. 1H) for 15–30 min. Two large dark brown meconia pellets occurred on each side of the central, mesolateral position of the host puparia (Fig. 1H) in the parasitoid prepupal stage. Prepupae were yellow-white with no organs visible through the cuticle (Fig. 1R). Prepupae completed development to black pupae in 24 h.

Black pupae were 0.587 ± 0.026 mm long and 0.330 ± 0.027 mm wide. Female pupae completed development in 4–6 days. Outlines of parasitoid head, eyes, wing pads, and legs were visible. Female parasitized *B. tabaci* pupae were differentiated from male parasitized pupae by having two meconial pellets compared with four for males (Fig. 1S). Pupae that face the venter of the host turn over to face the antero-dorsum central wall of the host. Prior to emergence, the black pupal cuticle of the parasitoid was shed revealing an orange body, red ocelli, reddish black eyes, and fully developed wings and legs (Fig. 1T). Adult emergence occurred through holes chewed in the *B. tabaci* cuticle by parasitoid adult in the anterio-dorsum. Adult parasitoid emergence holes were made in 20–30 min.

Unmated parasitoid females laid unfertilized eggs externally over immature female parasitoids enclosed within *B. tabaci* puparia (Figs. 1I–K). Before egg laying, parasitoids searched for the position of the host by probing movements with their ovipositors. We observed egg laying and successful male development in third

instar parasitoids (in dry environment), prepupae, and early-stage black pupae of female *E. bimaculata* and prepupa and early black pupa of *E. transvena* (Figs. 1I–K). The externally laid male egg laid by virgin adult female *E. bimaculata* was generally attached to prepupal and early pupal stages of either *E. bimaculata* or *E. transvena* or found free in the *B. tabaci* puparium (Figs. 1L and M). Eggs were sticky and averaged 0.081 ± 0.097 mm long and 0.047 ± 0.017 mm wide (Fig. 1M). Usually single egg was found to attached to the host but superparasitism was observed in all afore said stages. Development to first instars occurred in 3–4 days.

First instar male parasitoids had 13 segments and averaged 0.159 ± 0.009 mm long and 0.081 ± 0.002 mm wide. No segmentation was observed during early development. First instar development occurred in 3 days. At this stage, the host female parasitoid larva became paralyzed and growth ceased. Hyperparasitized larvae were observed feeding on the primary parasitoid (Fig. 1N).

Male second instar parasitoids (Fig. 1O) averaged 0.298 ± 0.009 mm long and 0.091 ± 0.009 mm wide. During the 2–3 days development to third instars, second instars consumed most of the anterior/posterior portions of their hosts (Fig. 1P). Third instars averaged 0.503 ± 0.101 mm long and 0.178 ± 0.002 mm wide. Development also occurred in 2 days, and the remains of the host were consumed (Fig. 1Q). Third instars had nine pairs of spiracles, sickle-shaped bodies, and whitish-gray colored mandibles. Mature third instar males deposited meconium in the *B. tabaci* puparium. Meconium appeared in the prepupa as brown pellets on either side of the central peripheral regions of the *B. tabaci* puparium (Fig. 1R). In hyperparasitized *E. bimaculata* (prepupa and early black pupa) two groups of meconial pellets were formed, i.e., one belonged to the primary female parasitoid and other to the male parasitoid (Figs. 1R and S). But in hyperparasitized *E. bimaculata* third instars only one group of meconial pellets occurred. Thereafter, males were distinguished from females by the occurrence of two meconial pellets in the female and four in the male (Figs. 1R and S). If *E. transvena* prepupa and early black pupa were hyperparasitized by *E. bimaculata* two groups of meconia were found. First group belonged to the female *E. transvena* on either side of the *B. tabaci* vasiform orifice and next group belonged to the male *E. bimaculata* on either side of the central peripheral regions (Fig. 1S). Usually two or four meconial pellets were found in hyperparasitized *B. tabaci* puparium, but interestingly we observed six meconial pellets in the same host (Fig. 1W). This occurred because a hyperparasitized larva was parasitized again with a second male egg (Fig. 1V) developed into male adult. This was an indication of tertiary parasitism by same parasitoid.

Male *E. bimaculata* pupal development was completed in 4–5 days. During pupal development, a distinct head, body segments and development of adult coloration, formation of reddish black eyes, wings, and red-colored ocelli were observed. Prior to emergence, the pupal cuticle was shed and adult coloration could be seen through *B. tabaci* puparium (Fig. 1T). Male pupae also faced the host venter and turned over to face the dorsal side of the *B. tabaci* puparium. The adult parasitoid chewed a hole in the host dorsal wall to escape.

3.2. Gel electrophoresis tests

3.2.1. Esterase

Fig. 2 shows the esterase-banding pattern of non-parasitized and *E. bimaculata* parasitized *B. tabaci* nymphs. The *B. tabaci* cassava strain had three esterase bands, two slow and one fast. The two slow moving bands showed different R_f values for the cassava strain (0.046 and 0.091). A fast moving light band with R_f value of 0.137 occurred in *B. tabaci* nymphs. Parasitized nymphs had two thick darkly stained bands, one slow moving and one fast moving light bands of R_f values 0.012 and 0.140, respectively (Fig. 2).

3.2.2. Malate dehydrogenase

MDH showed two fast moving bands with R_f values of 0.238 and 0.410 in non-parasitized *B. tabaci* nymphs. Parasitized *B. tabaci* nymphs had a very thick fast moving band of R_f value 0.235 (Fig. 3).

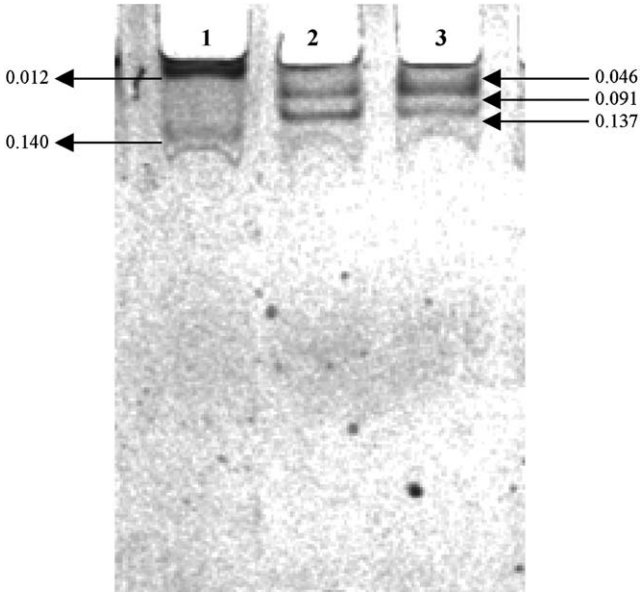

Fig. 2. Esterase-banding pattern of unparasitized and *E. bimaculata* parasitized *B. tabaci* nymphs. Lane 1, *E. bimaculata* parasitized *B. tabaci* nymph; lane 2, unparasitized *B. tabaci* nymph; and lane 3, unparasitized *B. tabaci* nymph.

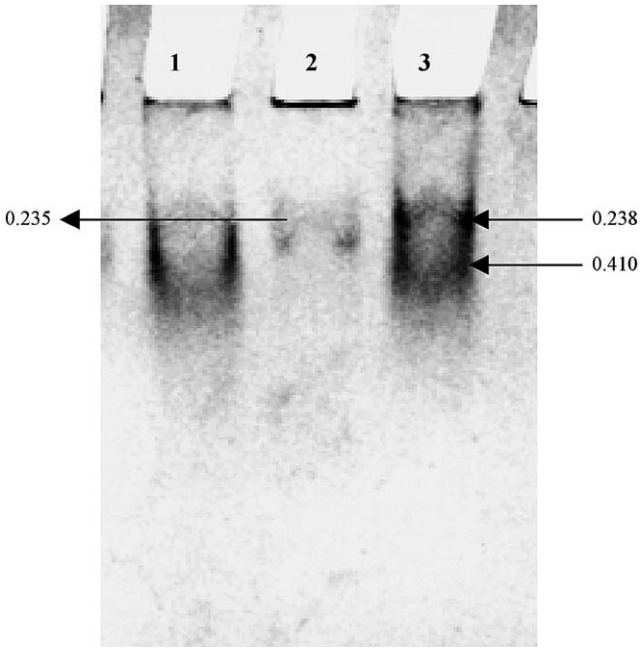

Fig. 3. Malate dehydrogenase-banding pattern of unparasitized and *E. bimaculata* parasitized *B. tabaci* nymphs. Lane 1, Unparasitized *B. tabaci* nymph; lane 2, *E. bimaculata* parasitized *B. tabaci* nymph; and lane 3, Unparasitized *B. tabaci* nymph.

3.2.3. Xanthine dehydrogenase

XDH had two fast moving, blue-colored bands with R_f values 0.116 and 0.147 for non-parasitized *B. tabaci* nymphs. Whereas *E. bimaculata* parasitized *B. tabaci* nymphs had a single fast moving thick dark colored band with an R_f value 0.206 (Fig. 4).

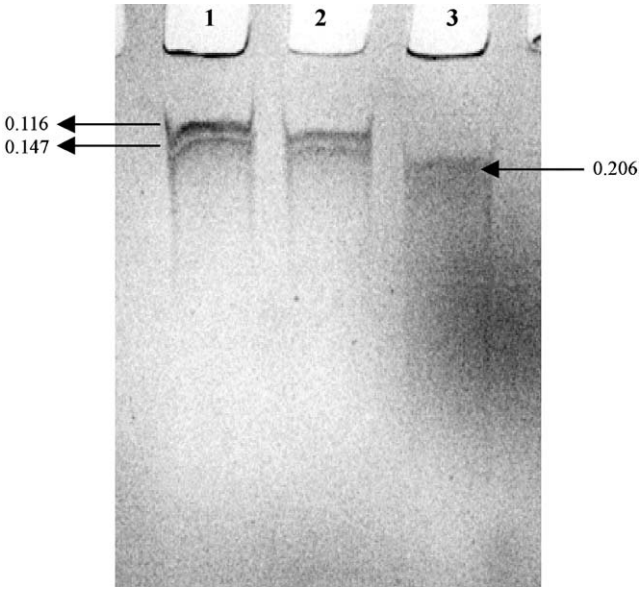

Fig. 4. Xanthine dehydrogenase-banding pattern of unparasitized and *E. bimaculata* parasitized *B. tabaci* nymphs. Lane 1, Unparasitized *B. tabaci* nymph; lane 2, unparasitized *B. tabaci* nymph; and lane 3, *E. bimaculata* parasitized *B. tabaci* nymph.

4. Discussion

Encarsia bimaculata, a recently described *B. tabaci* parasitoid, has been reported from Hong Kong, India, Philippines, Sudan, and USA (Heraty and Polaszek, 2000). *E. bimaculata* has a short life cycle. Our studies showed that *E. bimaculata* is a heteronomous autoparasitoid. Females develop as primary parasitoids on all stages of *B. tabaci* and males develop as hyperparasitoids on conspecific (*E. bimaculata*) and heterospecific females (*E. transvena*). *E. bimaculata* is developed similar to that of *E. transvena*, as reported by Antony et al. (2003) with few differences. Unlike from that of *E. transvena*, one interesting phenomenon we noticed in *E. bimaculata* was its male developed as tertiary parasitoid on conspecific male. In this case, female develop as primary parasitoid of *B. tabaci* and male develop as secondary parasitoid. When the secondary parasitoid reached its prepupal stage, it was being again hyperparasitized by *E. bimaculata* (Fig. 1S). Tertiary parasitism of heteronomous aphelinid has not been reported earlier (see in the reviews by Hunter and Woolley, 2001; Sullivan and Völkl, 1999). In *E. transvena*, Antony et al. (2003), reported that meconium occurred on each side of the host vasiform orifice. But for *E. bimaculata* meconium occurred on the mesolateral side of *B tabaci* pupae. The position of meconia in the host pupae differentiates *E. transvena* and *E. bimaculata* parasitization (Fig. 1S). We also observed that *E. bimaculata* produced six meconial pellets in the same host (Fig. 1W), when hyperparasitized larvae were parasitized with a second male *E. bimaculata* egg. This phenomenon has not been reported previously.

The differences in developmental times for male and female *E. bimaculata* might be due to its role as secondary parasitoid or because of competition with either conspecific or heterospecific larvae. Female. *E. bimaculata* eggs were large (0.168 mm), long and unhydropic (well provisioned with yolk) (after Hunter and Woolley, 2001). We observed that the developing embryo had an extraembryonic membrane, probably formed from polar bodies; thought to have a role in nutrient acquisition and immune suppression (see in Tremblay and Caltagirone, 1973), which was absent in male egg. As reported for *Encarsia porteri* (Hunter et al., 1996), first and second instar *E. bimaculata* males were enclosed within a transparent membrane, which was absent in females. Unlike males, females were found to move within the body fluid of the host. Male larvae were found to attach and feed on the body of the primary parasitoid. First instar *E. bimaculata* females had long tails, but unlike first instar *Encarsia pergandiella* (Gerling, 1983), the tails had no protuberance. *E. bimaculata* has a head notch containing a sickle-shaped, dark-gray colored mandible similar to that reported in *E. pergandiella* (Gerling, 1966) and *E. transvena* (Antony et al., 2003).

Larval morphology of *E. bimaculata* was exactly similar to that of *E. transvena*, since both come under the '*Encarsia strenua* group.'

As reported by Heraty and Polaszek (2000) we could observe that the *E. bimaculata* adult female was bigger than male, yellow colored with pale dark band of brown color across back of the head and between mesosoma and metasoma (Fig. 1U). Male coloration was much darker than female with entire metasoma brown (Fig. 1U).

Usually a single male egg was found attached to the primary host. But occasionally we observed superparasitism in all the host stages that we studied for male development. Gerling and Foltyn (1987) reported that superparasitism occurred when host discrimination efficiency was reduced. Wylie (1983) found that parasitoid larvae took longer to develop in superparasitized hosts than in singly parasitized hosts. Van Alphen and Visser (1990) suggested that having two (or more) eggs in a host might increase the probability of producing an offspring compared with single eggs. In our studies, superparasitism was highest in mass rearing cages with abundant host material. This agrees with Van Alphen and Visser (1990) and Visser (1993) that superparasitism occurred when females are not egg limited, the density of the searching females is high and many hosts are parasitized; also agrees with the report of Hunter and Goldfray (1995) that male eggs were produced only when hosts were abundant. Hence, the male/female ratio seems to depend on female abundance. Netting and Hunter (2000) reported that in *Encarsia formosa* (Gahan) female may kill eggs previously laid within the host by jabbing them with their ovipositor before ovipositing themselves. But, in *E. bimaculata* we could observe multiple ovipositional punctures, eggs and emerging first instars in the same host; ultimately we observed only one adult emergence. Since we found more than one emerging first instars in the same host, we did not believe in the phenomenon of ovicide in *E. bimaculata* as reported in *E. formosa* by Netting and Hunter (2000). Hence, an assumption has been drawn from this study that some physiological suppression may occur, which inhibit the egg development/other emerging instars. However, this needs further studies to clarify the statement.

As reported for *E. transvena* (Antony et al., 2001; Hunter and Kelly, 1998), *E. bimaculata* is a hyperparasitoid on conspecific as well as on heterospecific species, and may disrupt biological control programs if *E. bimaculata* are released with other primary parasitoids. Several earlier workers also reported the efficacy of biological control program was reduced if heteronomous hyperparasitoids were used together with primary parasitoids (see review by Sullivan and Völkl, 1999). But, the preference of *E. bimaculata* for conspecific hosts/heterospecific has not been well documented. Observations by Hunter and Goldfray (1995) and Hunter and Kelly (1998) with other autoparasitoids suggested that when primary and secondary host were available in the same leaf, females preferred to feed upon rather than oviposit in primary host, while converse was true in the case of primary and secondary hosts were on separate leaves. However, Hunter and Woolley (2001) in their review suggested that when primary and secondary hosts were intermixed in the same habitat, females search for both primary and secondary host simultaneously. Recent studies by Borgan and Heinz (2002) revealed that host selection and oviposition by heteronomous hyperparasitoids vary with the composition of host available for parasitization, and suggest a preference for heterospecific over conspecific secondary hosts. A survey of aphelinid parasitoids containing heteronomous hyperparasitoids and other primary parasitoids indicated that, in the majority of cases, the heteronomous hyperparasitoids were the most important species in the complex (Williams, 1996).

The advantage of the application of electrophoresis for endoparasitic detection was well illustrated (Atanassova et al., 1998; Wool et al., 1978). The methods have considerable advantage over either dissection or rearing to parasitoid emergence for identification. Earlier Wool et al. (1984) reported electrophoretic detection *Encarsia lutea* (Masi) and *Eretmocerus mundus* (Mercet). This is the first report, to our knowledge, of using electrophoresis to detect *E. bimaculata* parasitized and non-parasitized *B. tabaci* nymphs. Nymphal dissections in the laboratory are time consuming and require considerable experience. Counting black parasitized pupae also requires much time and fungal infections and dying parasitized pupae before mummification may confound accurate identifications. In contrast, the electrophoretic technique gives rapid results compared with other methods described and are also economical and dependable. Wool et al. (1984) reported that if parasitoids are found to have species-specific electrophoretic patterns, there is no need to wait until they emerge as adults for their identification, saving considerable space and efforts. In this study, we evaluated three isozyme systems to differentiate parasitized and non-parasitized *B. tabaci* nymphs. Lisha et al. (2003) reported two fast moving and a slow band for esterase in a non-parasitized *B. tabaci* (cassava strain). Earlier, several workers (Brown et al., 1995b; Burban et al., 1992; Costa and Brown, 1991; Liu et al., 1992; Prabhaker et al., 1987) reported three bands for esterase and two for MDH enzyme systems. Our studies showed the same banding patterns occurred for non-parasitized *B. tabaci*. But *E. bimaculata* parasitized *B. tabaci* had two fast moving bands for esterase and a single faint dark band for MDH. XDH showed two slow moving bands for non-parasitized *B. tabaci* nymphs and a thick fast moving band for parasitized *B. tabaci* nymphs. These banding patterns were

clearly distinguishable in the non-parasitized and *E. bimaculata* parasitized *B. tabaci* nymphs. Our 5- to 8-day-old nymphs, and parasitoid larvae could have been third instars. Hence, parasitized *B. tabaci* nymph-banding-patterns might be confused with third instar of *E. bimaculata*. Our study suggests that esterase, MDH, and XDH are useful for determining *B. tabaci* parasitism by *E. bimaculata*. Although it is possible to detect the presence of *B. tabaci* parasitoids inside the late larva and pupa of the host microscopically, electrophoresis might make it possible to detect them at earlier stages. Thus, this technique is definitely more reliable compared with dissections and counting of *E. bimaculata* parasitized *B. tabaci* nymphs. The technique may also be useful for other smaller insects.

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